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64. (New) The method of claim 1, wherein the target sequence is double-stranded and comprises inverted repeat structures that form a stem-loop and hybridize to the oligonucleotide primer under the polynucleotide amplification conditions.

65. (New) The method of claim 1, wherein the amount of the second polynucleotide is about ten fold to about ten thousand fold less than the target polynucleotide.

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## REMARKS

As an initial matter, the undersigned wishes to thank Examiner Sisson for courtesies extended during telephone interviews conducted on October 14, 1998 and October 28, 1998.

Applicant believes a brief overview of the invention would be helpful.

The present invention features methods for controlling amplification of two distinct polynucleotides during an amplification reaction. That control is generally achieved by limiting amplification of one polynucleotide while allowing amplification of another polynucleotide to proceed. The methods are especially useful for reducing amplification of a control polynucleotide while allowing amplification of a target sequence to proceed in the reaction.

In one embodiment of the invention, amplification of the control is limited by use of an oligonucleotide primer that hybridizes to the control and the target sequence but has a 3'-mismatch only on the control hybrid. That mismatch occurs because of sequence differences between the primer 3'-end and the control. The differences do not usually exist between the target sequence and the primer. Amplification of the control hybrid does not proceed optimally due to the 3'-mismatch. Thus in this example, the 3'-mismatch suppresses amplification of the control hybrid while target amplification proceeds. More particular methods of the invention provide additional amplification control by contacting the mismatched primer with exonuclease and digesting the mismatch. In this instance, amplification of the control accompanies digestion of the 3'-mismatch of the primer. These features of the invention provide many advantages

including giving a user substantial control over amplification of one polynucleotide relative to another in the reaction.

The claims have been amended to more precisely define the invention. For example, claim 1 has been amended to more specifically point out reaction conditions by including steps for *combining* amplification reagents and *subjecting* sample to *polynucleotide amplification conditions*. The claim has been further amended to more specifically point out the control facilitated by the 3'-mismatch under the polynucleotide amplification conditions recited in the claim.

Related amendments have been made to claims 2, 9, 25, and 39 where appropriate. In particular, the claims have been amended to specify that the methods employ polynucleotide amplification conditions to produce multiple sequences.

It is believed that the pending claims point out sufficient conditions under which improved amplification results are achieved by this invention.

Support for the amendments and new claims 59-65 can be found throughout the application including the drawings and claims as originally filed.

For example, support for the combining and subjecting steps of claim 1 can be found, e.g, on page 12, lines 11-15 and in the Examples. See also Figures 1-3.

Polynucleotide amplification conditions are well-known in this particular field and are described throughout the specification. Specific support for the conditions can be found, e.g., on page 30, line 18 to page 33, line 11 in which conditions for amplifying nucleic acids are disclosed. Polynucleotide amplification conditions are more particularly referenced in the specification as exponential or linear amplification. See page 30, line 20 and page 31, line 1. Specific exponential amplification is taught on pages 31-33 of the application and includes PCR, single-stranded polynucleotide amplification, LCR, NASBA, and Q-beta-replicase amplification.

See also pages 31-32, 64-68, and the Examples for more specific information about these and other standard exponential amplification techniques. Further disclosure relating to linear amplification can be found e.g., on page 33. Disclosure relating to other known polynucleotide amplification conditions can be found on pages 7-10 of the specification.

Specific support for 3'-mismatch language in claim 1 can be found, e.g, at page 11, lines 1-9 in which use of the mismatch to control amplification is disclosed. Further support for this language can be found on page 11, lines 15-18, page 12, lines 15-18, and page 16, lines 3-6 of the specification. See also page 20, line 2 to page 22, line 4 and page 38, line 21 to page 39, line 7, and Figures 1-3 for additional support.

New claims 59-65 more specifically point out control facilitated by the 3'-mismatch recited in claim 1.

In particular, new claims 59 and 60 more particularly reference the control by providing for contact and digestion of the 3'-mismatch with 3' to 5' exonuclease. Specific support for the new claims can be found e.g., on page 12, line 18 to page 13, line 3; page 14, lines 13-14; page 15, line 17 to page 16, line 2; page 17, line 5; and page 21, lines 6-8. See also page 43, line 20 to page 45, line 4, the Examples and Figures 1-3 for additional support.

New claim 61 features use of a modified oligonucleotide primer to help control digestion by 3' to 5' exonuclease. The claim is supported, e.g., page 13, lines 6-11, page 14, line 18 to page 15, line 2, page 16, lines 2-14, page 17, lines 5-10, and page 21, line 12 to line 19. See also pages 39-41, page 56, line 13 to page 58, line 17, and Figure 2 for additional support for the claim.

The method of new claim 62 more particularly references binding of the oligonucleotide primer to target polynucleotide. The claim is supported by disclosure at page 11, lines 10-14, for example.

New claims 63-64 reference more particular polynucleotide amplification conditions in which the target sequence is single-stranded (claim 63) or double-stranded (claim 64). Support for the claims can be found e.g., on page 32, lines 1-8, page 36, line 7 to page 37, line 3 in which various inverted repeat structures such as stem-loops are disclosed.

Specific support for new claim 65 can be found on page 65, line 18 to page 66, line 3.

It is submitted that the amendments to the specification and the claims, as well as the new claims do not introduce new matter.

The Oath/Declaration submitted with the CPA application on December 7, 1998 was objected to as follows:

> The oath or declaration is defective. A new oath or declaration in compliance with :7 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02. The oath or declaration is defective because Non-initialed and/or non-dated alterations have been made to the oath or declaration. See 37 CFR 1.52(c).

Office Action at 2.

This objection has been addressed by this submission. In particular, submitted herewith is a supplemental Oath/Declaration executed by the Applicant. The submission confirms Applicant's previous claim to the benefit of prior U.S. Provisional Application Number 60/010,948 filed on February 1, 1996.

The disclosure was objected to on grounds of informalities on pages 9, 10, 51 and 68. In formulating the objection, the position was taken that:

> The disclosure is objected to because of the following informalities: Pages 9, 10, 51, and 68 recite the serial number of several US patent applications; however, the status of these applications is not indicated. In some instances the cases recited have been abandoned in favor of a file-wrapper-continuation application. In such instances, it is suggested that the new serial number be provided and the current status of the continuation application be

provided. In other instances reference has been made to applications which have either been allowed or have become abandoned. While one may incorporate essential subject matter by reference to an issued US patent, such is not permitted with respect to a US patent application that is abandoned. Should any of the abandoned applications contain essential subject matter, applicant is urged to provide an amendment to the specification which brings that essential subject matter into the specification of the captioned application.

Appropriate correction is required.

Office Action at 2.

It is believed that the objection to the specification has been addressed by this submission.

The Office Action also took the position that:

The disclosure was also objected to on ground that:

The use of the trademark TRITON X-100 has been noted in this application. It should be capitalized wherever it appears and be accompanied by the generic terminology. Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

Office Action at 3.

The objection to the specification has been addressed by this submission.

Claims 1-59 stand rejected under §112, first paragraph as being based on a non-enabling disclosure. In formulating the rejection, the position was taken that:

The conditions and characteristics of the starting material are critical or essential to the practice of the invention, but not included in the claim(s) is not enabled by the disclosure. (cit. omitted). While claims 1, 2, 9, 25, 39, and 58 are each drawn to a related method, claim 1 is cited to exemplify the issue at hand. Claim 1, in Jepson format, states that the improvement comprises:

> forming said extension products in the presence of a second polynucleotide, to which said oligonucleotide primer hybridizes except for the 3'-end of said oligonucleotide primer, **under conditions** wherein the extension of said

oligonucleotide primer along said second polynucleotide is controlled relative to the extension of said oligonucleotide primer along said target sequence (emphasis added).

7. In order to achieve the requisite annealing/hybridization conditions whereby one does not have the 3'-end of the primer anneal to the second nucleic acid, it is imperative that the reaction mixture be accurately manipulated. As presently worded, the nucleotides of the second polynucleotide may have the same nucleotide residue sequence in that region corresponding to the primer annealing site. The claim is silent as to just how one would effect such annealing results and, by extension, the appropriate level of control, regardless of the similarity at the mend of the primer to the second polynucleotide. As set forth in Carrico, (US Patent 5,200,313) the extent and specificity of hybridization is affected by the following principal conditions:

1. The purity of the nucleic acid preparation.
2. Base compositions of the probe - G-C base pairs will exhibit greater thermal stability than A-T or A-U base pairs. Thus, hybridizations involving higher G-C content will be stable at higher temperatures.
3. Length of homologous base sequences- Any short sequence of bases (e.g., less than 6 bases), has a high degree of probability of being present in many nucleic acids. Thus, little or no specificity can be attained in hybridizations involving such short sequences. From a practical standpoint, a homologous probe sequence will often be between 300 and 1000 nucleotides.
4. Ionic strength- The rate of reannealing increases as the ionic strength of the incubation solution increases. Thermal stability of hybrids also increases.
5. Incubation temperature- Optimal reannealing occurs at a temperature about 05° - 30° C below the melting temperature for a given duplex. Incubation at temperatures significantly below the optimum allows less related base sequences to hybridize.
6. Nucleic acid concentration and incubation time- Normally, to drive the reaction towards hybridization, one of the hybridizable sample nucleic acid or probe nucleic acid will be present in excess. usually 100 fold excess or greater.
7. Denaturing reagents- The presence of hydrogen bond-disrupting agents, such as formaldehyde and urea, increases the stringency of hybridization.
8. Incubation- The longer the incubation time, the more complete will be the hybridization.
9. Volume exclusion agents- The presence of these agents, as exemplified by dextran and dextran sulfate, are thought to increase the effective concentrations of the hybridizing elements thereby increasing the rate of resulting hybridizations.

Office Action at pages 4-8.

Applicant respectfully disagrees with the rejection. Claims 1-57 fully satisfy the "how to make" and "how to use" requirements of 35 U.S.C. §112, first paragraph. Claim 58 has been cancelled.

The position was taken that "conditions and characteristics of the starting material are critical or essential to practice of the invention but are not included in the claim(s)". See the rejection above. This position raises two issues that are each addressed individually below.

It is alleged that critical or essential conditions for practicing the invention are not included in the claims. Applicant disagrees. Conditions for practicing the claimed methods are pointed out with enough precision to make and use the invention. For example, the claims reference specific amplification conditions, ie., polynucleotide amplification conditions. As would be understood from reading Applicant's disclosure, these conditions are specific and are well known to those in this particular field. Illustrative polynucleotide amplification conditions are abundantly referenced and discussed throughout Applicant's disclosure. Accordingly, there is no basis for the position that more specific conditions should be added to the claims.

It is stressed that use of the claimed methods is not limited to a specific polynucleotide amplification condition. As disclosed throughout the specification, the invention is fully compatible with many polynucleotide amplification conditions depending e.g., on desired results. For example, see the specification at page 10, lines 16-18 (disclosing that the invention broadly applies to amplification of polynucleotides) and page 30, line 18 to page 31, line 2 (referring to amplification as methods resulting in formation of polynucleotide copies). See also the previous discussion in which more specific polynucleotide amplification conditions were referenced.

The position is also taken that the claims must reference more specific starting material characteristics. Applicant again respectfully disagrees. Starting materials have been referenced in the claims with enough specificity for one in this particular field to make and use the invention. As would be understood from Applicant's disclosure, the claimed methods are compatible with a variety of starting materials suitable for the polynucleotide amplification

conditions. More particular starting materials are recited in claim 1 as polymerase and oligonucleotide primer. The primer is more specifically referenced as having a 3'-mismatch. Additionally specific primers are recited in claims 2, 9, 25, and 39 as having 1-10 nucleotides at the 3'-end that do not hybridize to template. Additionally specific starting materials are exemplified throughout the disclosure. See also the discussion above, the Detailed Description of the Invention, and the Examples for additional reference to suitable starting materials.

Accordingly, reconsideration and withdrawal of these grounds of rejection are respectfully requested.

Claims 1-58 have also been rejected under §112, first paragraph on grounds that primer annealing and control must be more specifically pointed to. See item 7 above.

Claim 58 has been cancelled. Applicant respectfully disagrees with the rejection as it pertains to claims 1-57. These claims recite conditions which are particular enough to make and use the invention.

The claimed methods recite polynucleotide amplification conditions that are well known to those working in this particular field. More specificity about the conditions is not needed to make and use the invention.

Additionally, the claims point out how control is to be achieved with ample precision. For example, claim 1 has been amended to show that the control is facilitated by the 3'-mismatch. As is apparent from Applicant's disclosure, the 3'-mismatch does not provide for optimal amplification. The control provided by the methods, ie., limiting amplification of one hybridized template (primer has the 3'-mismatch) and allowing amplification on another template ( no primer 3'-mismatch) is facilitated by the mismatch on the primer. See also the Summary of the Invention and particularly page 10, line 15 to page 11, line 9. Further specificity about the control is not needed to practice the invention.

Reconsideration and withdrawal of this ground of rejection are requested.

Carrico (U.S. Patent No. 5,200,313) has been cited to support the position that Applicant must point to more specific annealing and control conditions in the claims. The cited portion of Carrico (principal conditions 1-9) has been reviewed. It is submitted that this portion is not applicable to the present invention.

As understood, the Carrico patent discloses hybridization conditions for binding an immobilized or immobilizable polynucleotide probe. See Carrico's Abstract and also Figures 1 and 2 therein. As cited, Carrico does not teach polynucleotide amplification conditions for the bound probes. The cited portion of Carrico does not refer to any polynucleotide amplification conditions. Accordingly, the cited portion of the patent does not support the §112 rejection because it relates to different hybridization conditions then what are featured in the claimed methods.

As an illustration of significant differences between Carrico's hybridization schemes and those of the claimed methods, see, e.g., principal condition 7 ("Denaturing reagents"). As understood, this condition teaches adding disrupting agents to increase hybridization stringency. While this may be helpful in Carrico's hybridization schemes, it would not be a useful condition in the claimed methods. Use of such agents to increase hybridization stringency under polynucleotide amplification conditions would be detrimental. As an example, Carrico's denaturing agents would denature polymerase, thereby reducing or eliminating polynucleotide amplification achieved by the polymerase.

See also col. 12 of Carrico in which use of urea and formamide is taught. As understood, use of the agents in the way cited in the patent would hinder good use of the polynucleotide amplification conditions.

It is respectfully submitted that the cited portion of Carrico is used in the rejection out of context. Carrico's principle conditions are best understood in view of specific hybridization

schemes disclosed in the patent, e.g., at cols. 11 and 12, bridging paragraph. Carrico's principal conditions, as cited, immediately follow the specific hybridization schemes in the same paragraph. As cited, Carrico does not apply to the polynucleotide amplification conditions of the claims and cannot support the instant §112 rejection.

The hybridization conditions cited on page 6, lines 1-4 of the Office Action have been reviewed. It is not believed that the conditions are applicable to the claimed methods.

Claim 58 has been cited as exemplifying the ground of rejection set forth on page 6, lines 5-13. Claim 58 has been cancelled.

As discussed, claims 1, 2, 9, 25, and 39 have been amended to more particularly point out use of polynucleotide amplification conditions. These conditions are specific and fully support the hybridization and control intended. That is, the conditions allow hybridization of a 3'-mismatched primer to a polynucleotide for which amplification is to be limited. Amplification is suppressed (controlled) from the mismatched primer until that mismatch is digested.

In view thereof, reconsideration and withdrawal of the §112 rejection is requested.

Claims 1-58 stand rejected under §112, second paragraph as omitting essential steps. Applicants respectfully disagree. It is believed that the rejection is addressed by this submission.

The previous discussion is incorporated herein by reference. In view thereof, reconsideration and withdrawal of this rejection are respectfully requested.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned would appreciate the opportunity to do so. Early consideration and

allowance of this important case are earnestly solicited.

Respectfully submitted,

Date: 6/18/99

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